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(54) Title: METHOD FOR MEASURING TISSUE MORPHOLOGY

(57) Abstract

An apparatus for measuring one or more physical characteristics of material by means of optical radiation scattered by a tissue layer to determine, for example, the size of nuclei in a tissue layer. The apparatus includes fiber optic devices (10, 20, 30) to deliver and collect light from a tissue region of interest to determine, for example, whether the tissue is normal or precancerous.

METHOD FOR MEASURING TISSUE MORPHOLOGY

GOVERNMENT SUPPORT

The invention was supported, in whole or in part, by a
Grants No. P41RR02594 and CA53717 from the National
5   Institutes For Health.   The Government has certain rights
in the invention.

RELATED APPLICATIONS

This is a continuation-in-part of U.S. Serial No.
08/948,734 filed on October 10, 1997, the entire contents
10 of which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

Methods for diagnosis of cancer at an early stage are
essential for cancer prevention and therapy.   Many types of
cancers grow from epithelial tissues, which cover inner and

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outer surfaces of the human body. Many of these, for
example cancer in gastrointestinal tract, progress through
the stage of dysplasia. Dysplasia can be defined as
neoplastic tissue which is not malignant yet, but is
5 considered to be a precursor of malignancy. If diagnosed
at this stage, most tumors are curable. In the case of
gastrointestinal tumors, current methods of diagnosis are
based on endoscopy. However, dysplastic tissue is
frequently not endoscopically apparent. Thus, detection of
10 dysplasia in the gastrointestinal tract and other sites
often relies on sporadic sampling for this "invisible"
malignant precursor. However, sporadic biopsies have a
high probability of missing dysplastic changes. In some
cases the biopsy procedure is impossible.

15   Efforts toward a substitution for standard biopsies
have been made in order to provide accurate diagnosis of
cancerous tissue in different organs in vivo and in real
time. For this purpose, optical techniques that are non-
invasive do not require tissue removal and can be performed
20 in-vivo. Such methods provide information at the
microscopic level and can thus provide for the search for
very small sites which are likely to be missed by standard
biopsies. While most human organs can be diagnosed by
means of optical techniques, they are particularly
25 applicable to the tissues in human body lumene, since they
are easily accessible by optical probes, which can be

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inserted into one of the channels of a conventional
endoscopic tube.

SUMMARY OF THE INVENTION

The present invention relates to the use of light to
5   determine physical characteristics of a structured layer of
material, and in particular certain qualitative information
regarding the morphology of tissue structures using
scattered light. Both backscattered and transillumination
methods can be used, depending upon the thickness of the
10   material and the size and distribution of the structure
being measured. Examples of properties of materials that
can be measured include surface roughness, parasity,
cytometer measurements, or any material in which changes in
the refractive index of a material correspond to changes in
15   structures. This type of scattering spectroscopy can be
differentiated from absorption spectroscopy which is unable
to quantitatively measure particle morphology.

Despite extensive investigations, no reliable optical
technique to diagnose dysplasia in-vivo is known. One of
20   the difficulties resides in the fact that dysplastic
changes are limited to the uppermost epithelial layer,
which can be as thin as $20 \mu m$, a one cell layer that is
nearly transparent to optical radiation.

Tissue in the gastrointestinal tract, for example
25   (other hollow organs share the same features also), is
covered by a layer of cells called epithelium (from $20 \mu m$

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to 300μm thick depending on the part of the tract)
supported by relatively acellular and highly vascular loose
connective tissue, lamina propria, which can be up to
500μm in thickness and contains a network of collagen and
5   elastic fibers, and variety of white blood cell types.
Beneath the lamina propria there is a muscular layer,
muscularis mucosae, (up to 400μm thick) and another layer
of moderately dense connective tissue called submucosa
(400-600μm thick) containing many small blood vessels and
10   abundant collagen and elastic fibers. The overall
thickness of those layers is about 1mm. Since a
characteristic penetration depth of optical radiation into
biological tissue does not usually exceed 1mm, for a
preferred embodiment it is sufficient to limit measurements
15   of tissue by those layers.

Adenocarcinoma of the esophagus arises in metaplastic
columnar epithelial cells in the esophagus, termed
"Barrett's esophagus", which is a complication of long-
standing gastrointestinal reflux. In this condition, the
20   distal squamous epithelium is replaced by columnar
epithelium consisting of a one cell layer which resembles
that found in the intestines. Barrett's esophagus is
frequently associated with dysplasia which later can
progress to cancer. Trials of endoscopic surveillance of
25   patients with Barrett's esophagus have not resulted in a
reduction of esophageal cancer mortality. The most likely
explanation is that dysplasia occurring in the esophagus

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cannot be seen with standard endoscopic imaging and sporadic biopsy sampling is necessary. This procedure can sample only about 0.3% of the tissue at risk. Thus, there is tremendous potential for sampling error.

5   The application of optical techniques to diagnose dysplasia in Barrett's esophagus is limited by the fact that the primary alterations in the tissue occur in the epithelium which is one cell thick ($\sim 20\text{-}30\,\mu m$) while fluorescence or reflectance spectra are mostly formed in
10   deeper tissue layers. One of the most prominent features of a dysplastic epithelium is the presence of enlarged, hyperchromatic, and crowded nuclei. In fact, these changes in nuclei size and spatial distribution are the main markers used by a pathologist to diagnose a tissue specimen
15   as being dysplastic. No significant changes in other tissue layers is observed. Unfortunately, epithelium does not contain strong absorbers or fluorophores, and the thickness of the epithelium is relatively small and thus negligible. These make epithelium diagnosis in Barrett's
20   esophagus to be a difficult problem.

Diffuse reflectance spectroscopy can provide quantitative biochemical and morphological information for the analysis of biological tissue epithelium and the detection of precancerous lesions. Diffuse reflectance
25   spectra were collected from adenomatous colon polyps (cancer precursors) and normal colonic mucosa of patients undergoing colonoscopy. The data were analyzed using an

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analytical light diffusion system, which was measured on a physical tissue model composed of polystyrene beads and hemoglobin. Four parameters were obtained: hemoglobin concentration, hemoglobin oxygen saturation, effective

5   scatterer density, and effective scatterer size. Normal and adenomatous tissue sites exhibited differences in hemoglobin concentration and effective scatterer size. Thus, diffuse reflectance can be used to obtain tissue biochemical and morphological information *in vivo*.

10   A preferred embodiment of the present invention relates to a system of measuring a fine structure component in backscattered light from mucosal tissue which is periodic in wavelength. This structure is ordinarily masked by a diffusive background, which must be removed to

15   render it observable. The origin of this component is due to light which is Mie-scattered by surface epithelial cell nuclei. By analyzing the amplitude and frequency of the periodic structure, the density and size distribution of these nuclei can be extracted. These quantities are

20   important indicators of neoplastic precancerous changes in biological tissue, and the technique can thus provide a useful tool for observing such changes in patients undergoing endoscopy.

        The light that is incident on the thin layer at the

25   tissue surface is not completely randomized. In this thin region the details of the elastic scattering process can be preserved. Mucosal tissues, which line the hollow organs

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of the body, generally consist of a thin surface layer of
epithelial cells supported by underlying, relatively
acellular connective tissue.   In healthy tissues the
epithelium often consists of a single, well-organized layer

5   of cells with an endface diameter of about 10-20 µm and a
height of about 25 µm.   In cancerous and pre-cancerous
(dysplastic) epithelium cells proliferate, the cellular
layer often thickens and becomes more tightly packed, and
the cell nuclei enlarge and appear darker (hyperchromatic)

10   when stained.   This may indicate increased nucleic acid
density, hence increased refractive index.

A preferred embodiment of the invention utilizes a
broadband light source to illuminate the region of interest
in the tissue with optical radiation in the range between

15   350 and 700 nm.   A fiber optic probe can be used to deliver
and/or collect radiation from the tissue.   The system can
be used during endoscopy of a patient to optically survey a
body lumen within the patient and thereby eliminate the
need for removal of tissue for biopsy, or alternatively,

20   can be used to aid in locating tissue suitable for biopsy.

Backscattered light is preferably collected over a
small collection angle of between 2° and 12°, preferably in
the range between 3° and 8°.   When using an optical fiber
system to collect the scattered light fibers having a

25   numerical aperture between 0.05 and 0.22, and preferably
between 0.07 and 0.1 can be used.   Collection angles within

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this range reduce the level of background light without loss of the periodic component in the returning light.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram of a fiber optic probe
5   in accordance with the invention.

Figure 2 is an enlarged view of the distal end of an endoscope in accordance with the invention.

Figures 3A, 3B and 3C illustrate reflectance spectra from cell monolayers for normal colon cells ($\bar{R} = 0.46$);
10   T84 cells ($\bar{R} = 0.38$); (c) $BaSO_4$ diffusing plate ($\bar{R} = 1.0$)

Figure 4 illustrates nuclear size distributions from data of Figures 3A and 3B, respectively for normal colon cells; and T84 cells respectively. In each case, the solid line is the distribution extracted from the data, and the
15   dashed line is the distribution measured using light microscopy.

Figures 5A, 5B and 5C are reflectance spectra from Barretts' esophagus for diffuse reflectance from a normal site (solid line), a dysplastic site (dashed line), and the
20   model fit (thick solid line); for corresponding fine structures; and of resulting nuclear size distributions, respectively.

Figure 6 graphically illustrates a comparison of samples analyzed by standard pathology and the optical
25   methods in accordance with the invention.

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Figure 7 is a system used for *in vitro* tissue analysis in accordance with the invention.

Figure 8 is a process flow diagram illustrating a method of performing an optical diagnosis of tissue in
5 accordance with the invention.

Figure 9 is the molar extinction coefficient spectra (per heme group) of the oxygenated (thin line0 and deoxygenated (thick line) hemoglobin (21). Note the characteristic peaks at 415, 542, and 577 nm
10 (oxyhemoglobin-HbO_2) and at 430 and 555 nm (deoxyhemoglobin-Hb).

Figure 10 is the reduced scattering cross section spectra σ' (λ), calculated using Mie theory. Results are shown for four different diameters, d_s, (0.2, 0.6, 1.0, and
15 2.0 μm). The slope of the spectra is inversely related to the diameter. A refractive index of 1.4 was assumed for the scattering particles, and 1.36 for the surrounding medium.

Figure 11 shows the Diffuse reflectance spectra
20 measured on physical tissue models (thick line) corresponding to four different Hb concentrations: (a) 0.0 mg/dL, (b) 50 mg/dL, (c) 125 mg/dL, (d) 250 mg/dL. The analytical model predictions using the same optical parameters employed in the preparation of the physical
25 tissue models are also shown (thin line), with agreement between the analytical and the tissue model being very good.

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Figure 12 shows the typical normal and adenomatous polyp spectra (thick line), and best fits to the data using the model (thin line). Mie theory was used to approximate the reduced scattering coefficient used for the model fits

5   (see Figure 13).

Figure 13 shows the scattering spectra obtained from the data shown in Figure 12 (thin line), and best fits using Mie theory (thick line). The Mie theory fits assign an effective scatterer size d_s to the reduced scattering

10   spectra. The polyp is characterized by a larger effective scatterer size ($d_s = 1.5$ μm) as compared to normal mucosa ($d_s = 0.35$ μm).

Figure 14A-14D show parameters obtained from data analysis: (A) total Hb concentration. C_{Hb}, (B) Hb oxygen

15   saturation, α (C) effective scatterer density, p_s, and (D) effective scatterer size, d_s. The largest difference between normal mucosa (squares) and adenomatous polyps (stars) is observed in the total Hb concentration.

Figure 15 is a binary plot of the total Hb

20   concentration C_{Hb} vs. the effective scatterer size d_s,. The normal data tend to form a well defined cluster, while the adenomatous polyp data are marked by wider variation.

The foregoing and other objects, features and advantages of the invention will be apparent from the

25   following more particular description of preferred embodiments thereof, as illustrated in the accompanying drawings in which like reference characters refer to the

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same parts throughout the different views.   The drawings
are not necessarily to scale, emphasis instead being placed
upon illustrating the principles of the invention.

DETAILED DESCRIPTION OF THE INVENTION

5   A preferred embodiment of the invention involves the
use of a fiber optic system to deliver and collect light
from a region of interest to measure one or more physical
characteristics of a surface layer.   Such a system is
illustrated in Figure 1.   This system can include a light
10   source 50 such as a broadband source, a fiber optic device
10 for delivery and/or collection of light from the tissue,
a detector system 80 that detects the scattered light from
the tissue, a computer 120 having a memory 122 that
analyzes and stores the detected spectra, and a display 124
15   that displays the results of the measurement.   A lens 60
can be used to couple light from the source 50 into the
excitation fiber 30 of the probe 10.   A filter 110 and lens
system 90,100 can be used to efficiently couple collected
light to a spectrograph 70.   A controller 140 connected to
20   the data processing system 120 can be connected to a clock
and a pulser 150 that controls the light source 50.

          The distal end 15 of the probe 10 is illustrated in
Figure 2 where the central excitation fiber 30 is
surrounded by six peripheral collection fiber 20.   The
25   distal end of the device can be enclosed in an optical
shield 25 such as that described in U.S. Patent No.

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5,199,431, the entire contents of which is incorporated
herein by reference.   Other endoscopic devices can be used
such as an optical needle as described in the above
referenced patent or as described in U.S. Patent No.

5   5,280,788, the entire contents of which is also
incorporated herein by reference.

    The collection fibers 20 preferably have a numerical
aperture in the range of 0.05 to 0.22 in order to provide a
desired collection angle from the material being measured.

10 This aids in reducing background that is removed from
scattering spectrum without loss of the periodic component.

    The collection fibers can also be replaced or
supplemented by a distally mounted imaging sensor 35 such
as a charged coupled device or CMOS imager.   The sensor has

15 a pixellated structure that is sensitive to the different
colors contained in the scattering spectrum being recorded.
Further details regarding the use of a distally mounted
sensor can be found in U.S. Serial No. 08/745,509 filed on
November 12, 1996, the entire contents of which is

20 incorporated herein by reference.

    The backscattered light collected with this system can
be analyzed to determine certain physical characteristics
of epithelial tissue.   The relationship between the
collected light and the physical characteristics to be

25 determined using this light can be described as follows.

    Epithelial nuclei can be represented as spheroidal Mie
scatterers with a refractive index higher than that of the

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surrounding cytoplasm. Normal nuclei have a characteristic
diameter $l=4\text{-}7\mu m$. In contrast, dysplastic nuclei can be
as large as 20 μm in size, occupying almost the entire
cell volume. Thus, in the visible range, the wavelength
5    $\lambda<<l$, and the component of light scattered by the nuclei
will exhibit a periodicity with wavelength, the details of
which are determined by the nuclear size distribution. The
Van de Hulst approximation can be used to describe the
optical scattering cross section of the nuclei:

10    $$\sigma_s(\lambda,l)=\frac{1}{2}\pi l^2\left\langle 1-\frac{\sin(2\delta/\lambda)}{\delta/\lambda}+\left\langle\frac{\sin(\delta/\lambda)}{\delta/\lambda}\right\rangle^2\right\rangle,\qquad\qquad(1)$$

where $\delta=\pi l n_c(n-1)$, with n_c the refractive index of
cytoplasm and n the refractive index of the nuclei relative
to that of cytoplasm.

When a beam of light is incident on an epithelial
15   layer of tissue, a portion of this light is backscattered
from the epithelial nuclei, while the remainder is
transmitted to deeper tissue layers, where it undergoes
multiple scattering and becomes randomized. All of the
diffusive light which is not absorbed in the tissue
20   eventually returns to the surface, passing once more
through the epithelium, where it is again subject to
scattering from the cell nuclei. Thus, the emerging light
consists of a large diffusive background plus the component
of forward scattered and backscattered light from the
25   nuclei in the epithelial layer. For a thin slab of

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epithelial tissue containing nuclei with size distribution N(l) (number of nuclei per unit area (mm²) and per unit interval of nuclear diameter (μm)), the approximate solution of the transport equation for the reflectance
5   R(λ) collected by an optical probe with acceptance solid angle Ω_c is given by the following expression:

$$\frac{R(\lambda)}{R(\lambda)} = e^{-r(\lambda)} + 1 - \frac{e - r(\lambda)}{I_d(\lambda,s)_{\Omega_c}}\left\langle I_i\left(\lambda,-s'\right)p\left(\lambda,s,-s'\right)\right\rangle_{\Omega_l} + \left\langle I_d\left(\lambda,s'\right)_2\pi\right\rangle_{\Omega_c} \qquad (2)$$

where I_i $((\lambda, s))$ is the intensity of the incident light delivered in solid angle Ω_i, $I_d(\lambda,s)$ is the intensity of the
10   light emerging from the underlying tissue, and

$\langle I(s)\rangle\Omega = \int\limits_{\Omega} I(s)ds$ for any function I(s) and solid angel Ω,

with **s** a unit vector pointing outward from the tissue surface in an arbitrary direction. The quantity

$\overline{R}(\lambda) = \left\langle I_d(\lambda,s)\right\rangle_{\Omega_c} / \left\langle I_i(\lambda,s)\right\rangle_{\Omega_c}$ is the reflectance of the
15   diffusive background. The optical distance

$\tau(\lambda) = \int\limits_{o}^{\infty} \sigma_s(\lambda,1)N(1)dl$ and scattering phase function

$p(\lambda,s,s') = \frac{1}{\tau}\int\limits_{o}^{\infty} p(\lambda,l,s,s')\sigma_s(\lambda,l)N(l)dl$ both depend on N(l); for a

sphere, $p(\lambda,l,s,s')$ is determined by Mie theory. The first term in Eq. (2) describes the attenuation of the diffusive

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background, and the terms in brackets describe backscattering of the incident light and forward scattering of diffusive background by the epithelial cell nuclei, respectively.

5   For small Ω_c the forward scattering term in Eq. (2) can be expanded in $\tau(\lambda)$. Thus, $\left\langle \left\langle I_d(\lambda,s') \right\rangle_{2\pi} \right\rangle_{\Omega_c} / \left\langle I_d(\lambda,s) \right\rangle_{\Omega_c} \cong f_o + f_1 \tau / \tau_o$,

with $\tau_o = \pi/2 \int_o^\infty l^2 N(l)\,dl$. It is found numerically that $f_1 << f_0$ and that f_0 and f_1 are approximately independent of wavelength in the range of interest $\left(\lambda_{min} = 360 \; to \; \lambda_{max} = 685nm\right)$. Similarly,

10   for the backscattering term

$$\left\langle \left\langle I_i(\lambda,-s') p(\lambda, s-s') \right\rangle_{\Omega_i} \right\rangle_{\Omega_c} / \left\langle I_d(\lambda,s) \right\rangle_{\Omega_c} \cong b_o - b_1 \tau/\tau_o.$$ Note that in the

forward scattering contribution the first order term oscillates in phase with $\tau(\lambda)$ as required by the optical theorem, whereas for the backscattering contribution it is

15   out of phase. Thus, Eq. (2) reduces to

$$\frac{R(\lambda)}{\overline{R}(\lambda)} = e^{-\tau(\lambda)} + (1 - e^{-\tau(\lambda)})\left\langle f_1 + b_0 + (f_1 - b_1)\frac{\tau(\lambda)}{\tau_0(\lambda)} \right\rangle,   \tag{3}$$

which shows that the epithelial nuclei introduce a periodic fine structure component into the reflectance with a wavelength dependence similar to that of the corresponding

20   scattering cross section. Its periodicity is approximately proportional to nuclear diameter, and its amplitude is a

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function of the size and number of nuclei in the epithelial
layer. These quantities can be determined by analyzing the
reflectance, $R(\lambda)$.

As example of the effects described by Eq. (2),
5   elastic light scattering from normal T84 tumor human
colonic cell monolayers (10 and 15 sites respectively) was
measured and analyzed. The cells, approximately $15\mu m$
long, were affixed to glass slides in buffer solution and
placed on top of a $BaSO_4$ diffusing (and highly reflective)
10 plate. The $BaSO_4$ plate was used to approximate the diffuse
reflectance from underlying tissue. The diameters of the
normal cell nuclei generally ranged from 5 to 7 μm and
those of the tumor cells from 7 to $16\mu m$.

An optical fiber probe was used to deliver white light
15 from a xenon arc flashlamp to the samples and collect the
return reflectance signal, as shown in Figure 1. The probe
tip, 1 mm in diameter, consisted of a central delivery
fiber surrounded by six collection fibers, all of which
were covered with a 1 mm thick quartz optical shield. The
20 fibers were 200 μm core fused silica, NA=0.22

$\left(\Omega_i = \Omega_c = \pi NA^2\right)$. To eliminate specular reflection, the probe

was beveled at 17^0 to the normal. At the proximal end the
collection fibers were arranged in a line and imaged onto
the input slit of a spectrograph. A diode array detector
25 recorded the reflectance spectra from 360 to 685 nm.

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Figures 3A and 3B show the normalized reflectance $R(\lambda)/\bar{R}(\lambda)$ from normal and T84 tumor cell samples, respectively. Distinct spectral features are apparent. For comparison, the reflectance spectrum from the BaSO$_4$

5 plate by itself is also shown in Figure 3C. This spectrum lacks structure and shows no prominent features.

To obtain information about the nuclear size distribution from the reflectance data, Eq. (3) needs to be inverted. The nuclear size distribution, N(l), can then be

10 obtained from the Fourier transform of the periodic component of the optical distance $\tau - \tau_o \cong (1 - R(\lambda))/q$. The parameter q = 1 - b$_0$ - f$_0$ +2(b$_1$ - f$_1$) is associated with forward and backward scattering, and depends on the probe geometry and the angular distribution of the incident and

15 reflected light. In this particular example q≈0.15. By introducing the effective wavenumber k=2Πn_c(n-1)$/\lambda - k_0$, and $k_o = 2\pi n_c(n-1)/\lambda_{max}, K = 2\pi n_c(n-1)\langle \lambda_{min}^{-1} - \lambda_{max}^{-1}\rangle$ and we obtain,

$$N(1) \cong \frac{2}{ql\pi^2}\int\limits_{o}^{K}\left\langle\frac{R(k)}{\bar{R}(k)} - 1\right\rangle e^{ikl}(k + k_o)dk. \tag{4}$$

Equation (4) was used to analyze the data. In order to

20 remove spurious oscillations, N(l) was further processed by convolving it with a Gaussian filtering function. The solid curves in Figure 4 show the resulting

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nuclear size distributions of the normal and T84 cell
monolayer samples extracted from the spectra of Figures 3A
and 3B. A nucleus-to-cytoplasm relative refractive index
of $n=1.06$ and cytoplasm refractive index of $n_c=1.36$ were

5　used. The dashed curves show the corresponding size
distributions, measured morphometrically via light
microscopy. The size distributions can be approximated by
Gaussian distributions. The parameters for those are
presented in Table 1. The extracted and measured

10　distributions are in good agreement for both normal and T84
cell samples.

<u>Table 1</u>

	Normal Cells		Tumor T84 Cells	
	Mean Diameter (μm)	Standard Deviation (μm)	Mean Diameter (μm)	Standard Deviation (μm)
Microscopy	~6	~0.5	10.2	2.0
Spectroscopy	6.2	0.45	10.1	2.2

The periodic fine structure in diffuse reflectance of
esophagus and colon mucosa of human subjects can be
measured during gastroenterological endoscopy procedures.
In the case of Barretts' esophagus, in which the epithelium

20　consists of a thin monolayer of columnar cells similar to
those used in the cell culture experiments, data were
collected as in the cell culture studies. The optical
fiber probe is inserted into the biopsy channel of the
endoscope and brought into contact with the tissue surface.

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The methods described herein can also be used to measure structural properties of other GI tissue, tissues in the oral cavity, the cervix, the bladder, and skin.

The fine structure component, which is the scattering

5   signature of the cell nuclei, is typically less then 5% of the total signal and is ordinarily masked by the background of diffusely scattered light from underlying tissue, which itself exhibits spectral features due to absorption and scattering, as shown in Figure 5A. Its spectral features

10   are dominated by the characteristic absorption bands of hemoglobin and collagen scattering. In order to observe the fine structure, this background must be removed. The absorption length, μ_a^{-1}, ranges from 0.5 to 250 mm as the wavelength is varied, and the effective scattering length

15    $(\mu_s')^{-1}$ ranges from 0.1 to 1 mm. Thus, both scattering and absorption have to be taken into account in subtracting or removing the background signal.

To represent the background light incident on the tissue is assumed to be exponentially attenuated, and that

20   at any given depth, z, an amount of light proportional to the reduced scattering coefficient μ_a is scattered back towards the surface and further exponentially attenuated. Since light attenuation depends on both scattering and absorption, the attenuation coefficient is assumed to be

25   the sum of absorption coefficient μ_a and effective scattering coefficient $\mu_s^{(e)} = \beta\mu_s'$. The parameter β was

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determined by comparison with Monte Carlo simulations and more accurate models of light transport, and was found to be $\beta \cong 0.07$. Since light only penetrates ~1 mm into the tissue, most of the diffusely scattered return light is

5   confined to the mucosal layer.

The tissue is thereby represented as a two layer medium and neglected diffusely reflected light from the lower layer. The following approximate expression for the diffusive light from underlying tissue impinging on the

10   epithelial cell layer is then obtained:

$$I_d(\lambda, s) = F(s) \langle I_l(\lambda, s) \rangle_{\Omega i} \frac{1 - \exp[-(\mu_s^{(e)} + c\mu_a)L]}{1 + c(\mu_a / \mu_s^{(e)})} \tag{5}$$

with F(s) being a Lambertian function describing the angular dependence of light emerging from mucosal layer, L a parameter representing the thickness of the mucosal

15   layer, and L a parameter representing the thickness of the mucosal layer, and c, the concentration of hemoglobin, which we find to be the main absorber relative to that of collagen, which is responsible for light scattering. Because both oxygenated and deoxygenated hemoglobin are

20   present, the total hemoglobin absorption is represented as

$\mu_a = (1-a)\mu_{(a)}^{(hb)} + \alpha\mu_a^{(hbO_2)}$ with oxygen saturation parameter

$\alpha(0 \leq \alpha \leq 1)$.

Figure 5A shows the reflectance spectra from two Barretts' esophagus tissue sites, both independently

diagnosed by standard pathological analysis to indicate (1)

normal and (2) precancerous (i.e. low grade dysplasia). As

can be seen, the differences in these unprocessed spectra

are small. To analyze them, Eq.(5) was first fit to the

5   broad features of the data by varying the parameters c, a

and L. As seen in Figure 5A, the resulting fits are quite

accurate. After removing this diffuse background structure

by calculating $R(\lambda)/\overline{R}(\lambda)$, the periodic fine structure is

seen in Figure 5B. Note that the fine structure from the

10   dysplastic tissue site exhibits higher frequency content

than that from the normal site. Equation (4) was then

employed to extract the respective nuclear size

distributions, yielding Figure 5C. The difference between

normal and dysplastic tissue sites is evident. The

15   distribution of nuclei from the dysplastic site is much

broader than that from the normal site and the peak

diameter is shifted from ~7 μm to about ~10 μm. In

addition, both the relative number of large nuclei (>10μm)

and the total number of nuclei are significantly increased.

20   Based on computer analysis, the uncertainty of the

above method in extracting nuclear size information is

estimated to be from 5% to 30%, depending on the noise

level and accuracy of the model. The distributions were

calculated using the same refractive index for both normal

25   and dysplastic nuclei. This is not entirely correct,

inasmuch as in stained histological sections dysplastic

nuclei appear hyperchromatic, which may be indicative of an

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increase in refractive index. Thus, the relative number of
large nuclei in the distributions measured from dysplastic
sites may be slightly overestimated.

The ability to measure nuclear size distribution *in*
5   *vivo* has valuable applications in clinical medicine.
Enlarged nuclei are primary indicators of cancer, dysplasia
and cell regeneration. In addition, measurement of nuclei
of different size can provide information about the
presence of particular cells, and can thus serve, for
10 example, as an indicator of inflammatory response of
biological tissue. This suggests that different
morphology/pathology in the mucosal layer gives rise to
distinct patterns of nuclear distributions.

The physical characteristics that have been found to
15 be useful to differentiate between Barrett's non-dysplastic
and dysplastic epithelium were the total number of nuclei
and the percentage of large nuclei ($l > 10$ µm). A
comparison of pathological analysis of samples with the
optical analysis thereof provided the plot (total number of
20 nuclei vs. percentage of nuclei with a diameter large that
10 µm) in Figure 6. From those 50 sites, the cumulative
sensitivity and specificity, for this analysis were 83% and
100% respectively. The study had a positive predictive
value is 100%. The points indicated by n's were either
25 normal or inflamed and those indicated by d's were
displastic. A percentage in the range of 20-30% was found

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to be an accurate diagnostic for this type, with 25% being used in this particular example.

A preferred embodiment of a spectrograph system 300 employed for the collection of backscattered spectral data
5 from excised tissue samples using a spectrograph and a charge coupled device (CCD), CMOS or other integrated solid state imaging array is illustrated in Figure 7.

System 300 can use a broadband light source or tunable laser 314 for irradiating a sample 46. Source 314
10 generates a beam 316 which is directed by mirror 318 through focusing optics 320 to impinge on sample 46 mounted on a scattering substrate 325 and behind a transparent window 321. The beam was focused on the sample at an angle of incidence. The collection angle 330 can be determined
15 by an aperture or collimator 340 and is between 2 and 12 degrees, preferably between 3 and 8 degrees.

A portion of the scattered light 322 emitted by sample 46 was collected by collecting optics 324 a small angle relative to the incident light. In another preferred
20 embodiment the angle of incidence and collection can be along a single common axis. Collecting optics 324 collimates and F/matches the collected light for the spectrograph 310. Prior to entering the entrance slit of the spectrograph 310, the collected light was passed
25 through a series of filters 326 which attenuated the unwanted scattered component of the collected light.

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Figure 8 illustrates generally a process 400 for collecting and analyzing the scattering spectrum from a material of interest such as tissue. The method can be performed both *in vitro* using a microscopy system or a

5   color imaging system as shown in Figure 7, or *in vivo* on a patient. The illuminating light 402 from a source can use radiation in the range of 300nm-1200nm, including the infrared range. After collecting 404 and detecting 406 radiation, the diffuse background 408 can be removed and

10   the desired characteristics calculated 410. These results can be used to provide a diagnosis of the region of interest.

A preferred embodiment of the present invention employs an analytical method based on data collection with

15   an optical fiber probe with fixed delivery and collection geometry. Data analysis uses light diffusion, but rather than directly apply the model to analyze tissue spectra, a correspondence is established between the diffuse reflectance spectra and a physical tissue representation

20   composed of scatterers and absorbers with known optical properties. By analyzing spectra from this tissue model using the analytical formulation, a calibration system is provided which is simple to invert and accurately predicts the concentrations of the scatterers and absorbers. Once

25   the calibration is established, the method is applied to tissue spectra.

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The following relates to the application of spectral analysis to mucosal surfaces of tissues *in vivo*. In this example adenomatous colon polyps are measured, which are precursors of colon cancer. These polyps are a form of

5   colonic dysplasia and are histologically similar to visually undetectable flat dysplasia, and are readily detectable. The results are correlated with standard histological examination, and can be used for early detection of disease and, most importantly, exemplify how

10   this spectroscopic method can be applied *in vivo* to obtain morphological and biochemical information.

Diffuse reflectance spectra were collected *in vivo* from adenomatous polyps on 13 patients undergoing routine colonoscopy. Data were collected simultaneously with

15   multi-excitation fluorescence spectra: a xenon-arc flashlamp with 10 μs pulse duration and an average input energy of 4Jj/pulse was used as white light source. An imaging spectrograph dispersed the collected, light and a gated diode array detector was employed for light detection

20   in the 360-685 nm spectral range. A 12 μsec gate synchronized with the lamp pulse was used to minimize background from endoscopic illumination. The detector was controlled by a PC notebook computer, where the data were transferred and stored.

25   Light was delivered and collected using an optical fiber probe which was advanced through the accessory channel of the colonoscope and brought into contact with

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the tissue. The probe consisted of a central optical fiber
for light delivery, surrounded by six concentric fibers for
light collection. All fibers had 200 μm core diameter and
NA=0.22. The probe tip was fitted with a quartz shield
5   approximately 1.5 mm in length and diameter, which provided
a fixed delivery/collection geometry with uniform circular
delivery and collection spots in the form of overlapping
cones with approximate radii of r_d=0.35 mm and r_c=0.55 mm,
respectively. The tip was beveled at an angle of 17
10   degrees, to eliminate unwanted specular reflections from
the shield/air interface.

The diffuse reflectance spectrum of a 20% by volume
$BaSO_4$ powder suspension was used as a reference, to take
into account the spectral characteristics and overall
15   intensity of the xenon lamp. The probe was immersed in the
suspension and a reference diffuse reflectance spectrum was
recorded prior to collection of each data set. Tissue
spectra were calibrated by dividing by this reference
spectrum. Diffuse reflectance spectra were measured from a
20   few different sites on every adenomatous polyp and from
corresponding sites in the surrounding normal mucosa.
Single-pulse excitation was used in order to avoid motional
artifacts. The polyps were than removed and examined
histologically, while the normal mucosa sites were not
25   biopsied.

To represent diffuse reflectance, biological tissue
was approximated as being a homogeneous semi-infinite

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turbid medium with reduced scattering and absorption
coefficients μ_s' (λ) and μ_a' (λ), respectively (λ is the
wavelength of light). Part of the incident light is
absorbed in the tissue, while the non-absorbed part is
5   subject to multiple scattering and eventually emerges from
the surface as diffuse reflectance. A certain fraction of
this return light is collected by the probe, while the
remaining part escapes undetected. The amount of the light
collected depends on the optical properties μ_s' (λ) and μ_a'
10   (λ), as well as on the probe diameter, r_p. Because r_p is
finite, it serves as a scale length, enabling μ_s' (λ) and
μ_a' (λ) to be determined separately.

To characterize light collection by an optical probe,
knowledge of the spatial and angular resolution of the
15   diffuse reflectance on the surface of the tissue is
required. Using the method of images to implement the
diffusion approximation to the radiative transfer equation
the diffuse reflectance radial density, R $(\lambda,$ $r)$, at a
distance r from the point of incidence of the light on the
20   surface of a semi-infinite turbid medium is given by

$$R(\lambda,r) = \frac{z_0 \mu_s'}{4\pi\mu_s' + \mu_s}\left\{(\mu+\frac{l}{r_1})\frac{e^{-\mu r_1}}{r_1^2} \div (1+\frac{4}{3}A)(\mu+\frac{1}{r_2})\frac{e^{-ur_2}}{r_2^2}\right\}, \tag{6}$$

where $\mu = (3\mu_a(\mu_a - \mu_s'))^{1/2}, z_o\dfrac{1}{\mu_s' + \mu_a}$

and $r_1 = \left(z_0^2 + r^2\right)^{1/2}, r_2 = \left(z_0^2(1+\frac{2}{3}A)^2 + r^2\right)^{1/2}$

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The parameter A depends on the refractive index n of the
medium (A=1 for n=1 and A>1 for n>1). A reasonable
assumption for the average refractive index of colon tissue
is n≅ 1.4 so that A ≅ 3.2.

5   To find the total light collected by the probe, Eq. 6
must be integrated over the spatial extend of the delivery
and collection areas, characterized by radii r_d and r_c,
respectively. Assuming the incident light intensity to be
uniform over the entire delivery area, the diffuse
10   reflectance R_p (λ) collected by the probe is given by

$$R_p(\lambda) = \frac{1}{r_d^2} \int_o^{r_c} r dr \int_o^{2\pi} d\phi \int_o^{r_d} R(\lambda, \bar{r} - \bar{r}') r' dr' , \qquad (7)$$

with $| \bar{r} - \bar{r}' | = \left(r^2 + r'^2 - 2rr' \cos\Phi \right)^{1/2}$. The integrals is Eq. 7 can be

evaluated numerically. However, to obtain a simple

analytical expression for R_p (λ), we assume point delivery

15   of light $(r_d = 0)$ and collection over a circular spot of

radium r_c, we then obtain:

$$R_p(\lambda) = 2\pi \int_o^{r_c} R(r) r dr$$

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$$= \frac{\mu_s'}{\mu_s' \div \mu_a} \left\{ e^{-uz_o} \times e^{-\left(1+\frac{1}{3}A\right)uz_o} - z_o \frac{e^{-ur'}}{r_1'} - \left(1+\frac{4}{3}A\right)z_o \frac{e^{-\mu r_2'}}{r_2'} \right\} \qquad (8)$$

with $r_2' = \left(z_o^2 + r_c^2\right)^{1/2}, r_2' = \left(z_o^2\left(1+\frac{2}{3}A\right)^2 + r_c^2\right)^{1/2}$

The optimal value of r_c was found by calibrating Eq. 8 on
physical tissue models with known optical properties. The

5   advantage of using Eq. 8 is that it is much easier to
invert than Eq. 7, which requires numerical integration.
Eq. 8 gives the diffuse reflectance at the perpendicular
direction to the tissue surface. This is acceptable
because light is only collected by the optical probe at

10   directions with a maximum deviation of approximately 10
degrees from the direction perpendicular to the surface.
Eq. 8 can be used to analyze the diffuse reflectance
spectra collected by the probe. From these measurements of
the tissue spectra, hemoglobin is the only significant

15   light absorber in colon tissue in the visible range of the
spectrum, and is encountered in both oxygenated and
deoxygenated forms. The light absorption properties of
both forms have been studied and their molar extinction
coefficient spectra $\epsilon_{HbO2}(\lambda)$, $\epsilon_{Hb}(\lambda)$ are shown in Figure 9.

20   Oxyhemoglobin absorption presents a maximum at 415 nm and
two secondary maxima at 542 and 577 nm, while
deoxyhemoglobin has a maximum at 430 nm and only one

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secondary maximum at 555 nm. The total absorption
coefficient, $\mu_j (\lambda)$ is given by

$$\mu_u(\lambda) = 2.3C'_{hb}\left(\alpha\varepsilon_{hb0_2}(\lambda)+(1-\alpha)\varepsilon_{hb}(\lambda)\right) \quad,$$ (9)

where $\quad \alpha = \dfrac{C_{hb0_2}}{C_{hb0_2}+C_{hb}} \quad$ is the Hb oxygen saturation parameter,

5   C_{hb0_2} and C_{Hb} are the concentrations of oxy and deoxy Hb,

respectively, and $C'_{hb} = C_{hbo_2} + C_{Hb}$ the total concentration
of HB.

Determination of C_{Hb} and α was performed in the
following way. For a given tissue spectrum, $R_p(\lambda)$, initial

10 values were assigned (typically $C_{Hb} = 0.0$ and $\alpha = 0.5$).
Eq. 8 was then numerically inverted to find $\mu_s'(\lambda)$, which
exhibited residual spectral features of HB absorption. The
parameters C_{Hb} and α were then modified, and the process
was repeated recursively until $\mu_s'(\lambda)$ exhibited a smooth

15 spectral shape with the Hb spectral features absent. In
this way, $\mu_s'(\lambda)$ was simultaneously determined with C_{Hb} and
α.

Table 2

	Model Parameter	Normal	Adenomatous Polyp
20			

Total Hb Concentration C_{Hb} (mg/dL)	13.6 ± 8.8	72.0 ± 29.2
Hb Oxygen Saturation α	0.59 ± 0.08	0.63 ± 0.10
Effective Scatterer Density ρ_s (x10^8 mm^{-3})	9.2 ± 7.5	3.5 ± 4.0
Effective Scatterer Size d_s (mm)	0.56 ± 0.18	0.94 ± 0.44

The above procedure is based on the assumption that $\mu_s'(\lambda)$ is a relatively smooth function of the wavelength λ, which analysis confirms, as will become evident from the discussion below. In general, $\mu_s'(\lambda)$ is the sum of contributions from the various tissue scatterers. Unfortunately, detailed information about these individual scatterers is not available. Therefore, $\mu_s'(\lambda)$ was written as

$$\mu_s'(\lambda) = \rho_s \sigma'(\lambda), \tag{10}$$

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with ρ_s the *effective* scattering density and σ' (λ) the *effective* reduced scattering cross section, i.e. the tissue scattering properties are modeled in an average way, as if tissue contained a single well-defined type of scatterer.

5   In general, σ' (λ) depends on the refractive index, shape and size of the scatterer, as well as on the refractive index of the surrounding medium. For a spherical scatterer with diameter d_s, σ' (λ) can be calculated numerically using Mie scattering theory.

10   Figure 10 shows such a calculation of σ' (λ). Note that the slope of σ' (λ) depends on d_s in a simple way i.e. the slope is inversely related to scatterer size. In this way it was possible to assign a scatterer size to every σ' (λ) and hence to every μ_s' (λ) in the range d_s = 0.2-2.0

15   μm. For example, by inspecting μ_s' (λ) of normal colon mucosa, previously reported based on *in vitro* measurements, it can be seen that they correspond to a scatterer size d_s $\cong$0.35 μm. Once d_s was determined, the density p_s was by dividing μ_s' (λ) by σ' (λ). In summary, for each tissue

20   diffuse reflectance spectrum four parameters were obtained, C_{Hb}, α, p_s and d_s.

         In calculating the results shown in Figure 10, the scatterer and the surrounding medium refractive indices were assumed similar to those likely to be found in tissue.

25   Reasonable approximations are a refractive index of approximately 1.4 for the scatterers and 1.36 for the surrounding medium. These values can be justified by

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noting that the lower bound for the tissue refractive index
is set by the refractive index of water, which is
approximately equal to 1.33 in the visible range, and the
upper bound is set by the maximum refractive index reported
5   for soft tissue, which is around 1.45. Such values are
consistent with those generally observed for soft
biological tissues.

     The physical tissue representation served as a method
for confirming the applicability of the various
10   approximations made in developing Eq. 8. The samples
consisted of mixtures of spherical microparticles with Hb
in various concentrations. The model established that
these mixtures simulated the optical properties of tissue
samples. In addition, the range of validity of the
15   analytical model in terms of the optical properties was
established, and the optimal value for the parameter r_c,
Eq. 8. was determined.

     Polystyrene bead suspensions in de-ionized water
(Polysciences, Inc.) were used to simulate tissue light
20   scattering, and Hb solutions prepared from lyophilized
human Hb (Sigma, H0267) were used to stimulate absorption.
The scattering properties of the beads were calculated
using Mie theory. The bead diameter was 1.07 μm, and the
refractive index n_p of polystyrene was given by the
25   expression of $n_p = 15607 + 10002/ \lambda^2$ (26), (λ in nm), and
the polystyrene concentration was 0.625% by volume. The
reduced scattering coefficient varied from approximately

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1.7 mm^{-1} at 400 nm. This spectral dependence is similar to that shown in Figure 10 with d_s = 1.0 μm, with the difference that the slope is larger due to the larger refractive index of polystyrene.

5   Since light absorption by polystyrene is negligible in the visible range, absorption was solely due to oxyhemoglobin. The size of the physical-tissue model was approximately 3x3x0.5 cm, simulating the flat geometry of normal colon mucosa. Additional geometries were

10   investigated such as a cylindrical geometry with 1 cm depth and 0.5 cm diameter, to simulate the geometry of the polyps, but no significant spectral differences were found. The range of optical parameters used was chosen based on the previously reported independent measurements of colon

15   tissue optical parameters performed in vitro.

      Figure 11 shows diffuse reflectance spectra measured on the physical tissue model with various concentrations of Hb vs. the analytical model predictions, which were obtained by setting r_c = 0.45 mm in Eq. 3. This was

20   determined to be the optimal value for r_c, which was kept fixed throughout the entire data analysis. It was also found that the parameter $Z_0 = 1/(0.6\mu_d + \mu_s')$ improved the agreement between the analytical model and the physical tissue model data, especially for large values of

25   absorption. The physical tissue model spectra shown in Figure 11 are very similar to the tissue spectra presented below. This fact serves as indirect confirmation of the

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assumptions made in developing the model, regarding tissue
scatterers (modeled as microspheres) and tissue absorption
(attributed to Hb).

The analytical model was found to accurately predict
5   the physical tissue model spectra for Hb concentrations in
the physiological range of interest. For Hb concentrations
lower than 100 mg/dL, the deviation between the two models
was always smaller than 10% over the entire wavelength
range. The largest deviation occurred when absorption
10 became comparable to scattering. This is shown in curve
(d) of Figure 11, near the peak of Hb absorption, around
415 nm. The Hb concentration was in this case 250 mg/dL
which corresponds to an absorption coefficient of
approximately 5 mm^{-1} at 415 nm, while the scattering
15 coefficient was approximately 2.8 mm^{-1} at the same
wavelength.

Figure 12 shows typical diffuse reflectance spectra
from one adenomatous polyp site and one normal mucosa site.
Significant spectral differences are readily observable,
20 especially in the blue region of the spectrum, where the Hb
absorption valley around 420 nm is the prominent spectral
feature. This valley is much more prominent int he polyp
spectrum, which also shows a continuous decrease in
intensity starting from the red end (~700 nm) and moving
25 toward the green region (~500 nm) of the spectrum, while in
the same range the normal mucosa spectrum shows a steady
increase in intensity. The secondary absorption dips of

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oxyhemoglobin (542 and 577 nm) are much more prominent in
the adenomatous polyp spectrum, indicating increased Hb
concentration. According to the model analysis the normal
mucosa spectrum was characterized by Hb concentration C_{Hb} =
5   22.5 mg/dL, while the corresponding value for the
adenomatous polyp was about 6 times higher (C_{Hb} = 165
mg/dL). The Hb saturation was found to be 0.65 ± 0.05, and
0.55 ± 0.05 respectively.

Figure 13 shows the scattering spectra μ_s' (λ)
10   calculated from the data shown in Figure 12, along with the
best Mie theory fits. The main difference between the
spectra of the two tissue types is observed in the spectral
slope, which corresponds to different effective scattering
sizes, d_s = 1.5 μm for the polyp and d_s = 0.35 μm for
15   normal mucosa. The values for the scatterer densities were
found to be p_s = 15x10^8 mm^{-3} for the normal mucosa, and p_s =
1.3x10^8 mm^{-3} for the adenomatous polyp. Figure 12 also
shows the best analytical model fits to the data, using Eq.
8. The model accurately describes the data, despite the
20   dramatic differences noted in the spectral shape between
the two tissue types and the fact that μ_s' (λ) is
approximated assuming a homogeneous distribution of
spherical scatterers. The deviation between the data and
the model in typically smaller than 10% for most of the
25   wavelength range.

Figures 14A-14D shows the calculated values of the
four parameters for all tissue sites studied: (A) total Hb

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concentration C_{Hb} (B) Hb oxygen saturation α, (C) effective
scatterer density p_s and (D) effectiveness scatterer size
d_s. Adenomatous polyps were clearly characterized by
increased Hb concentration, while there were no observable

5   differences in terms of the Hb oxygen saturation. The
effective scatterer density was in general lower in
adenomatous polyps, and the effective scatterer size
larger, even though there was significant overlap of these
parameter distributions between the two tissue types.

10 Table 2 summarizes the results shown in Figures 14A-14D by
giving the average values and the standard deviations for
each parameter.

     Figure 15 shows a plot of the Hb concentration C_{Hb},
vs. the effective scatterer size d_s. These two parameters

15 are shown together in order to summarize and illustrate the
differences found in the scattering and absorption
properties of normal mucosa and adenomatous polyps in the
colon. Note that the normal mucosa data tend to form a
cluster, while the adenomatous polyp data are separated,

20 and characterized by a wider spread and irregular
distribution, in both the effective scatterer size and the
Hb concentration.

     A methodology has been described which provides
quantitative information about colon mucosal tissues *in*

25 *vivo*, based on diffuse reflectance measurements. The main
components of this methodology are (a) data collection
through an optical fiber probe with a fixed

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delivery/collection geometry, (b) an analytical model for data analysis based on light diffusion, and (c) a physical tissue model for calibration of the analytical model.

The use of an optical probe with fixed geometry
5   enables consistent data collection, and independent determination of the scattering and absorption. Modeling of the probe geometry is facilitated through the use of the parameter r_c, which also defined the probe's sensitivity to absorption. A probe with large r_c will render the spectra
10   collected more sensitive to tissue absorption as compared to a probe with a smaller r_c. In Figure 4, the secondary Hb absorption peaks are barely noticeable in the normal mucosa spectrum: a probe with larger r_c would make these features more prominent.

15   The analytical diffusion theory model is amenable to numerical inversion and successfully describes the tissue data after calibration on the physical tissuemodel composed of polystyrene beads and oxy hemoglobin. Other researchers have already used variants of the analytical model
20   described here, in the study of diffuse reflectance from tissues and physical tissue models in the IR range. The present study is, to our knowledge, the first to apply the model in the visible range, to *in vivo* data from human mucosal tissue, measured with an optical fiber probe. By
25   working in the visible rather than in the IR, light penetration was restricted to approximately 0.5 mm, i.e. the mucosa layer where precancerous changes occur. Using

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the model, we have shown that it is possible to obtain
quantitative information about the tissue studied, such as
Hb concentration, Hb oxygen saturation, effective scatterer
size, and effective scatterer density. The results show
5   that diffuse reflectance provides a tool for quantitative
analysis of mucosal tissue surfaces *in vivo*.

The basic approximation made in the model is that Hb
is the only significant absorber in colon mucosa in the
visible range of the spectrum. Even though this assumption
10 lacks direct confirmation, the fact that the data strongly
exhibit the characteristic features of Hb absorption,
clearly indicates that Hb is a major absorber. In
addition, the spectra measured on the tissue physical
model, in which Hb was the only absorber, closely resemble
15 the tissue spectra.

In a similar way, tissue scattering was modeled as due
to homogeneous spherical scatterers with known refractive
index. The approximation permits a quantitative
characterization in terms of two basic parameters, the
20 effective scatterer size and the effective scatterer
density. These parameters provide an estimate of the
average scattering properties. The fact that the spectra
measured on physical tissue models reproduce the actual
tissue spectra, serves again (as in the case of Hb), as an
25 indirect confirmation of the validity of this
approximation.

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Data analysis showed that adenomatous colon polyps were characterized by increased Hb concentration. It is known that tumors and cancerous tissues exhibit increased microvasculature, and hence increased blood content. Using morphometry and vascular casting in combination with scanning electron microscopy techniques. Precancerous tissues such as adenomatous polyps of the colon are also characterized by increased microvascular volume the above in agreement with these reports in terms of observing the increased Hb concentration. Others report increased microvascularity of the colon mucosa associated with Crohn's disease and ulcerative colitis. Even though we did not study these tissue types, the technique employed here could prove useful for the *in vivo* study of such diseases.

The Hb oxygen saturation was found to be approximately 60%, on average, for both normal mucosa and adenomatous polyps. This result is reasonable, because the measurements were essentially performed in the capillary network of the mucosa, where oxygen is transferred from Hb to tissue. Hb oxygen saturation drops within the capillaries from approximately 97% (arterial blood) to about 40% (venous blood) with the measured values (around 60-70%) appropriately placed int he middle of this range. The fact that there were no differences observed between the two tissue types, can probably be attributed to the fact that adenomatous polyp metabolism is not significantly altered, so as to introduce changes in Hb oxygenation,

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which is probably related to the disturbed metabolism of such tissues. The technique presented here could be used *in vivo* for the study of tumors on the surfaces of hollow organs, or for the study of other tissue types when

5 knowledge of Hb saturation is needed.

    An intrinsic differentiation in the scattering properties between the two tissue types was observed. For adenomatous polyps, the average effective scattering size was larger, and the average effective scatterer density was

10 smaller, as compared to normal mucosa. There are a number of hypotheses identifying contributions to scattering from various microstructures, both extracellular such as collagen fibers, and intracellular such as mitochondria and cell nuclei. One model provides contributions to light

15 scattering from intracellular structures is increased in adenomatous polyps because cells occupy a higher volume ratio. In addition, the submucosa, in which collagen is more densely packed is usually located too far away from the polyp surface in order to be able to contribute to

20 light scattering. This can explain the lower scatterer density in adenomatous polyps, provided that intracellular scatterers are larger than extracellular ones, on average.

    EQUIVALENTS

    While this invention has been particularly shown and

25 described with reference to preferred embodiments thereof, it will be understood by those skilled in the art that

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various changes in form and details may be made therein

without departing from the spirit and scope of the

invention as defined by the appended claims. Those skilled

in the art will recognize or be able to ascertain using no

5   more than routine experimentation, many equivalents to the

specific embodiments of the invention described

specifically herein. Such equivalents are intended to be

encompassed in the scope of the claims.

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CLAIMS

What is claimed is:

1.   A method of measuring a size of a structure in a layer
      of tissue comprising:
5   directing radiation onto a region of interest in
      the layer of tissue;
              collecting radiation from the tissue;
              detecting the collected radiation; and
              determining a size of a structure within the
10   tissue layer.

2.   The method of Claim 1 further comprising determining
      if the region of interest includes dysplastic tissue.

3.   The method of Claim 1 further comprising directing
      radiation onto the tissue using a fiber optic probe.

15 4.   The method of Claim 1 further comprising collecting
      the radiation from the tissue with a fiber optic
      probe.

5.   The method of Claim 1 further comprising determining
      an average nuclear size of nuclei within the region of
20   interest.

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6.   The method of Claim 1 further comprising measuring a
      diameter of a tissue nucleus within the region of
      interest.

7.   The method of Claim 1 further comprising measuring a
5   periodic component of an intensity of radiation from
      the tissue as a function of wavelength.

8.   The method of Claim 7 further comprising determining
      the size of a nucleus in the tissue from the periodic
      component.

10   9.   A method of optically measuring tissue comprising:
               directing radiation onto a region of interest in
      tissue to be measured;
               collecting radiation from the tissue; and
               measuring a periodic component of collected
15   radiation as a function of wavelength to determine a
      physical characteristic of the tissue.

10.   The method of Claim 9 further comprising determining
      if the region of interest includes dysplastic tissue.

20   11.   The method of Claim 9 further comprising directing
      radiation onto the tissue using a fiber optic probe.

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12.   The method of Claim 9 further comprising collecting the radiation from the tissue with a fiber optic probe.

13.   The method of Claim 9 further comprising determining
5   an average nuclear size of nuclei within the region of interest.

14.   The method of Claim 9 further comprising measuring a diameter of a tissue nucleus within the region of interest.

10   15.   The method of Claim 9 further comprising collecting radiation with an endoscope, the endoscope having an imaging sensor at a distal end of the endoscope.

16.   The method of Claim 9 further comprising determining a density of nuclei in the tissue from the periodic
15   component.

17.   A method of determining a presence of dysplasia in tissue comprising:
          directing radiation onto a region of interest in an epithelial layer of tissue;
20   collecting backscattered radiation from the tissue;

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detecting the collected radiation with a
detector; and

determining the presence of dysplasia in the
region of interest of the tissue.

18. The method of Claim 17 further comprising determining
if the region of interest includes dysplastic tissue.

19. The method of Claim 17 further comprising collecting
radiation in the range of 350 nm to 700 nm.

20. The method of Claim 17 further comprising collecting
the radiation from the tissue with a fiber optic
probe.

21. The method of Claim 17 further comprising determining
an average nuclear size of nuclei within the region of
interest.

22. The method of Claim 17 further comprising measuring a
diameter of a tissue nucleus within the region of
interest.

23. The method of Claim 1 further comprising measuring a
periodic component of an intensity of radiation from
the tissue as a function of wavelength.

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24.   The method of Claim 7 further comprising determining
        the size of a nucleus in the tissue from the periodic
        component.

25.   A method of optically measuring a material comprising:
5   directing radiation onto a region of interest in
        the material tissue to be measured;
                collecting radiation from the material;
                detecting a scattering spectrum from the
        collected radiation; and
10   measuring a periodic component of collected
        radiation as a function of wavelength to determine a
        physical characteristic of the material.

26.   The method of Claim 25 further comprising directing
15   radiation onto the material using a fiber optic probe.

27.   The method of Claim 25 further comprising collecting
        the radiation from the material with a fiber optic
        probe, the probe having an optical fiber with a
        numerical aperture in a range of 0.05-0.25.

20 28. The method of Claim 25 further comprising determining
        an average nuclear size of nuclei within the region of
        interest.

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29. The method of Claim 25 further comprising measuring a number of particles per unit area within the region of interest.

30. An apparatus for optically measuring tissue
5   comprising:

    a radiation source that illuminates a region of interest in tissue to be measured;

    an optical system that collects radiation from the tissue;

10 a detector system that senses the collected radiation; and

    a data processor that determines a periodic component of detected radiation as a function of wavelength to determine a physical characteristic of
15 the tissue.

31. The apparatus of Claim 30 further comprising a broadband light source that generates light in a range of 350-700 nm.

20 32. The apparatus of Claim 30 further comprising a fiber optic probe that couples the source to the tissue.

33. The apparatus of Claim 30 further comprising a fiber optic probe that collects the light in a collection angle between 2 and 12 degrees.

SUBSTITUTE SHEET (rule 26)

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34. The apparatus of Claim 33 wherein the probe is
     insertable in an endoscope.
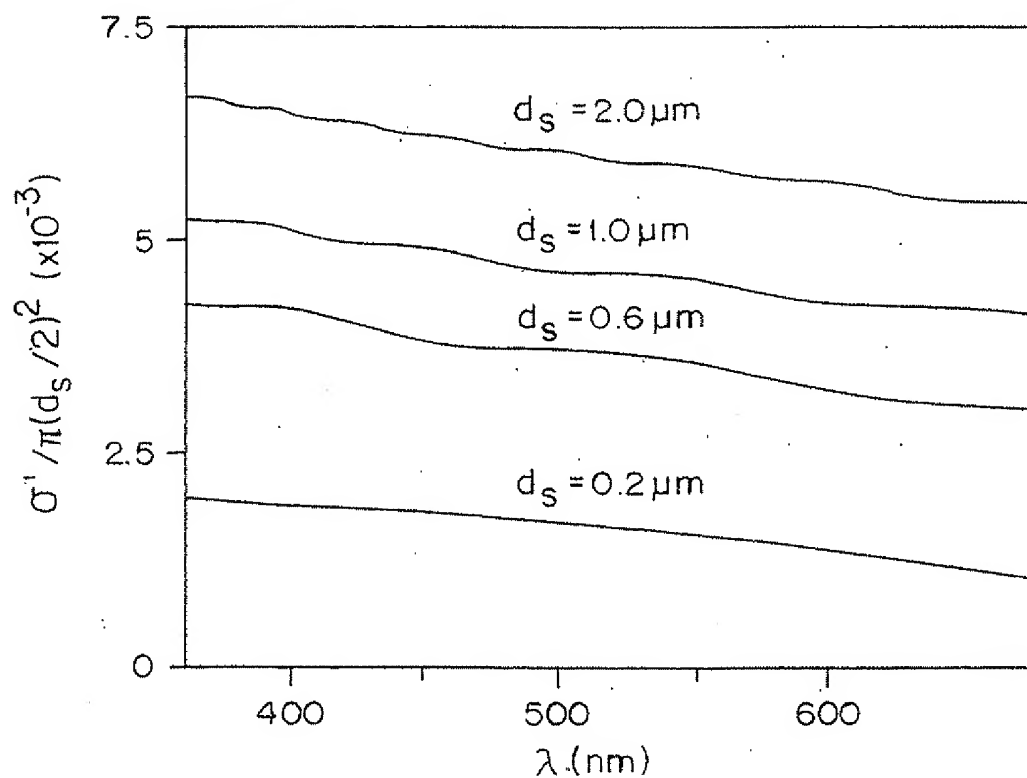
1/14

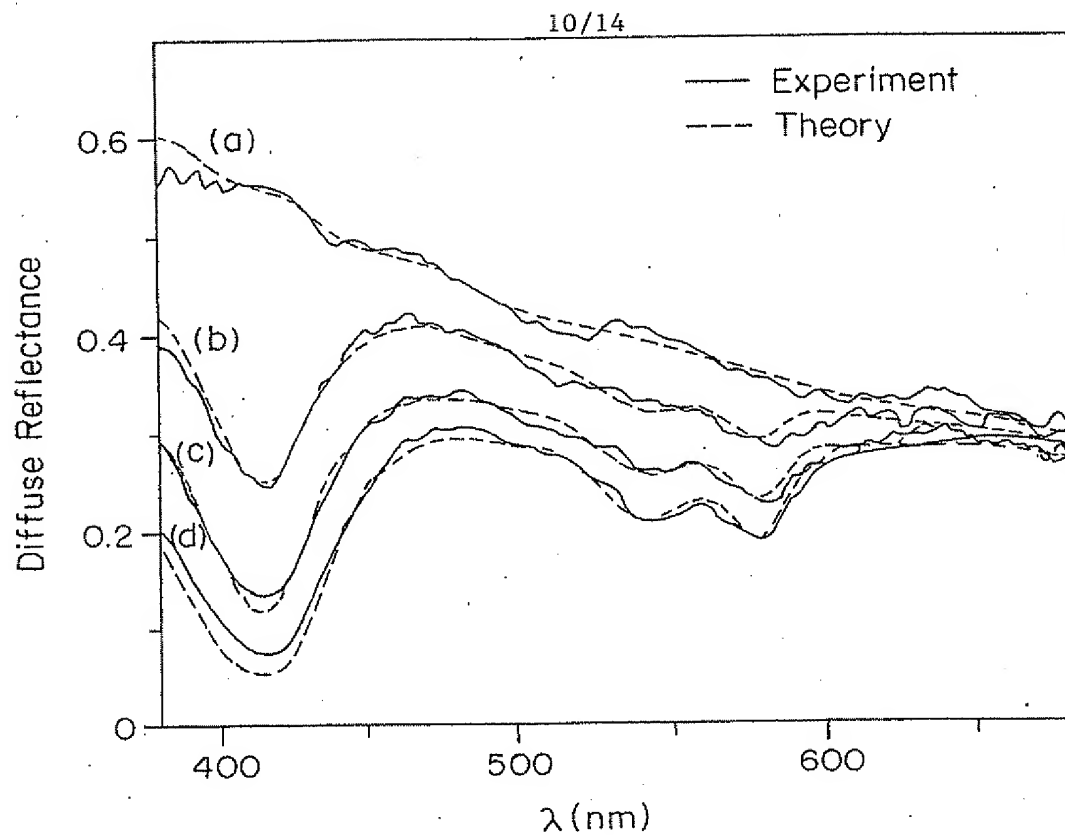
FIG. 1

FIG. 2

SUBSTITUTE SHEET (rule 26)

FIG. 3A

FIG. 3B

SUBSTITUTE SHEET (rule 26)

FIG. 3C

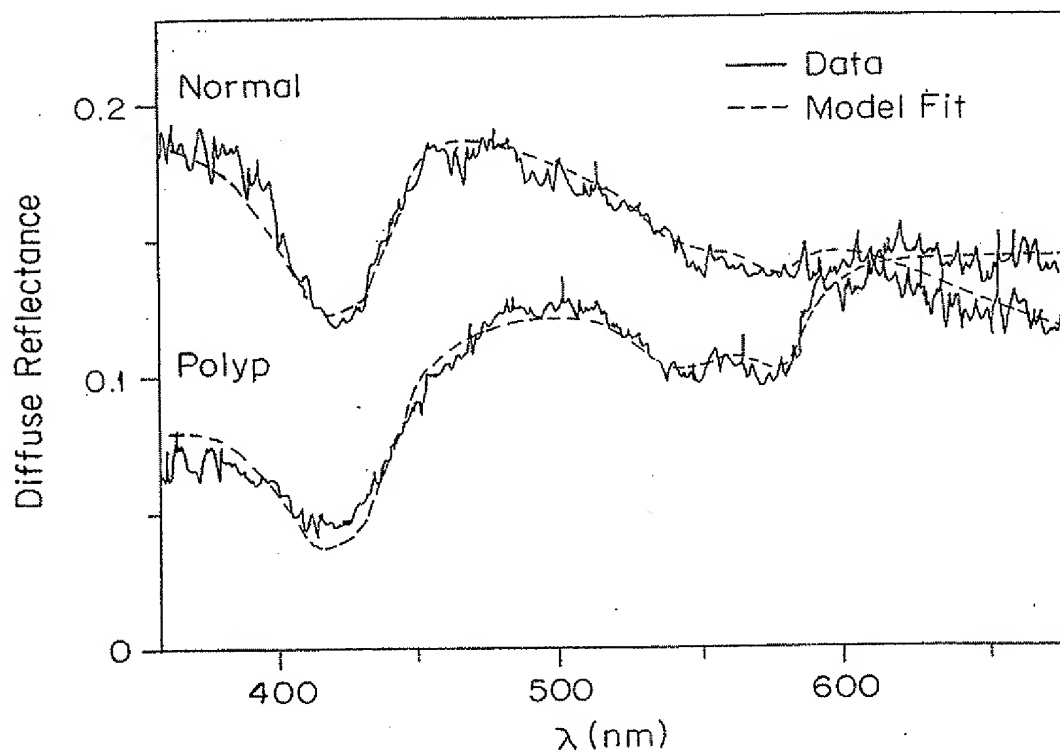FIG. 4

SUBSTITUTE SHEET (rule 26)

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FIG. 5A

FIG. 5B

SUBSTITUTE SHEET (rule 26)

FIG. 5C

FIG. 6

BNSDOCID: <WO　　　9918845A1 I >

FIG. 7

Illuminating a region of
interest of tissue or
material with radiation
having a wavelength be-
tween 300nm and 1200nm ⎯ 402

400 ⎯

Collecting radiation from
the tissue or material in
response to the illumin-
ating radiation at a col-
lection angle between 2°
and 12° ⎯ 404

Detecting the collected
radiation and generating
a spectral representation ⎯ 406

Removing a diffuse spec-
tral background from the
spectral representation to
provide the periodic spec-
tral structure ⎯ 408

Determining a physical
characteristic of the ma-
terial from the periodic
spectral structure such as
the particle size distribu-
tion or cell nucleus diam-
eter ⎯ 410

FIG. 8

FIG. 9

FIG. 10

FIG. II

FIG. I2

FIG. 13

12/14
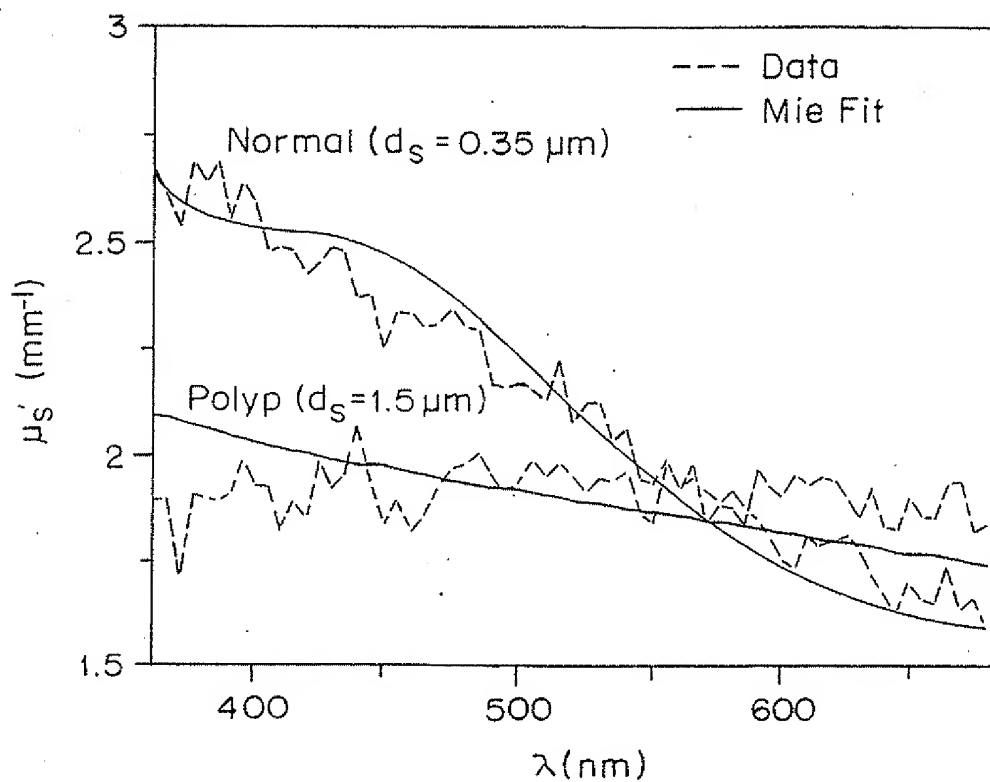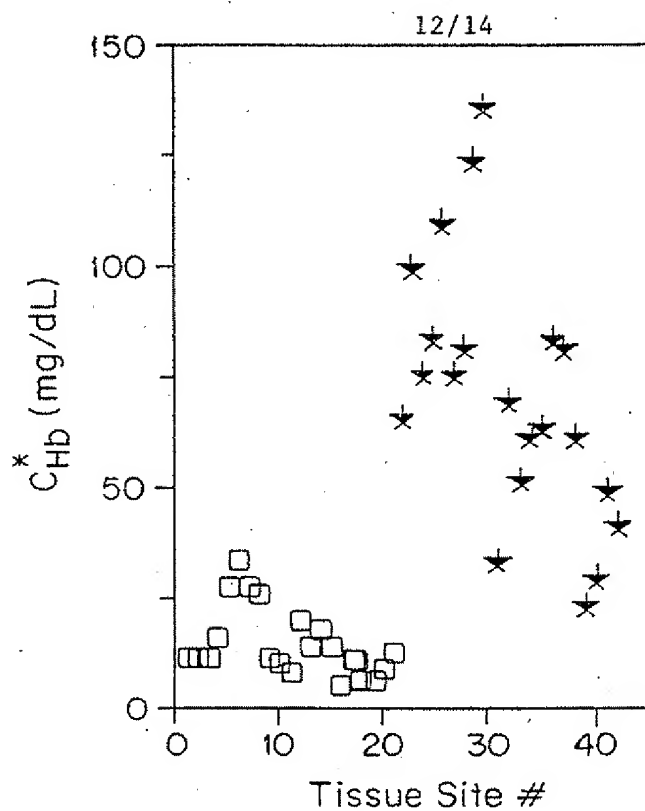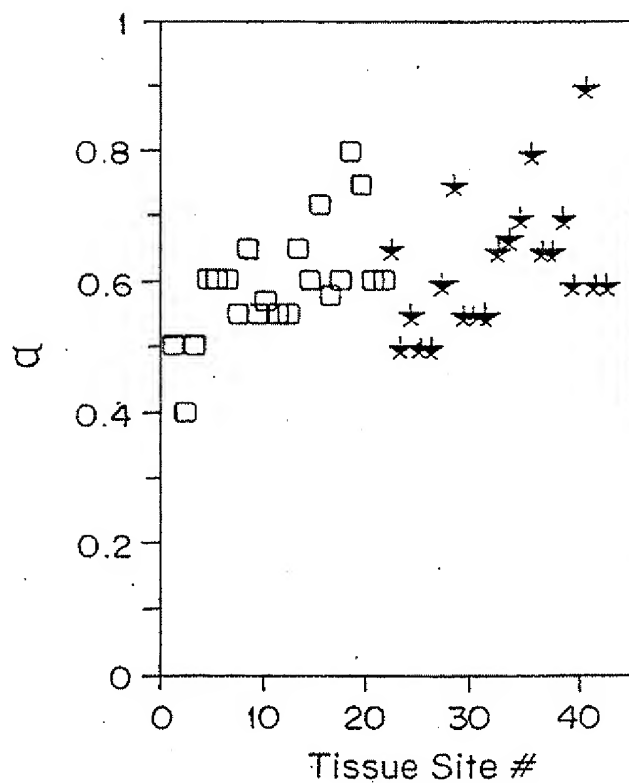

FIG. 14A

FIG. 14B

SUBSTITUTE SHEET (rule 26)
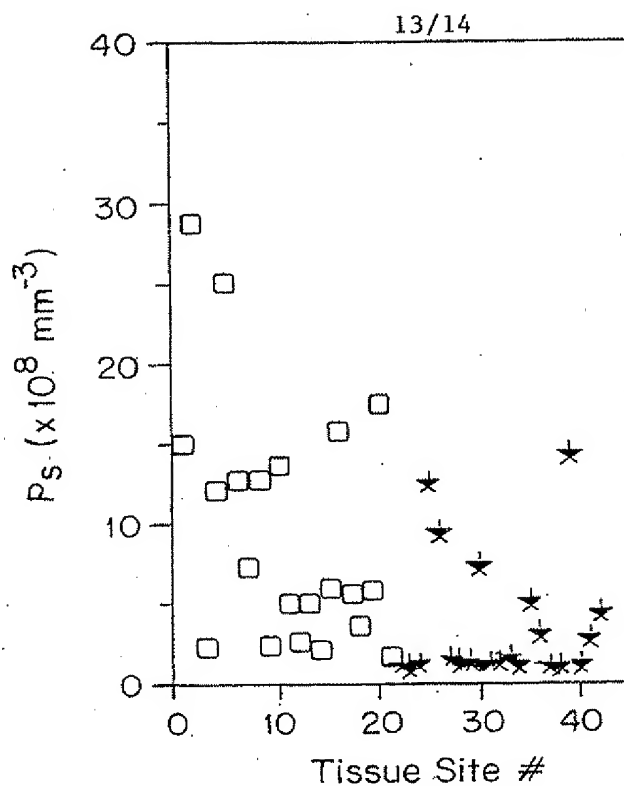
FIG. 14C
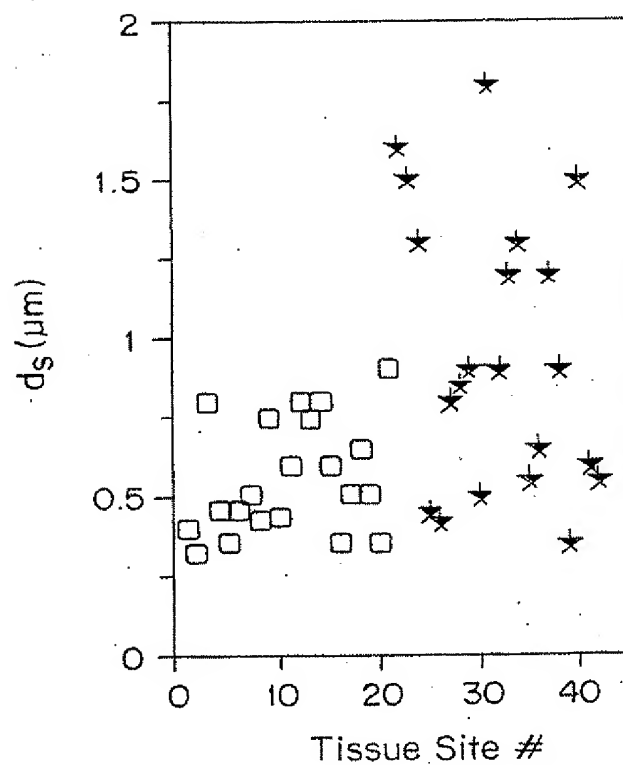
FIG. 14D
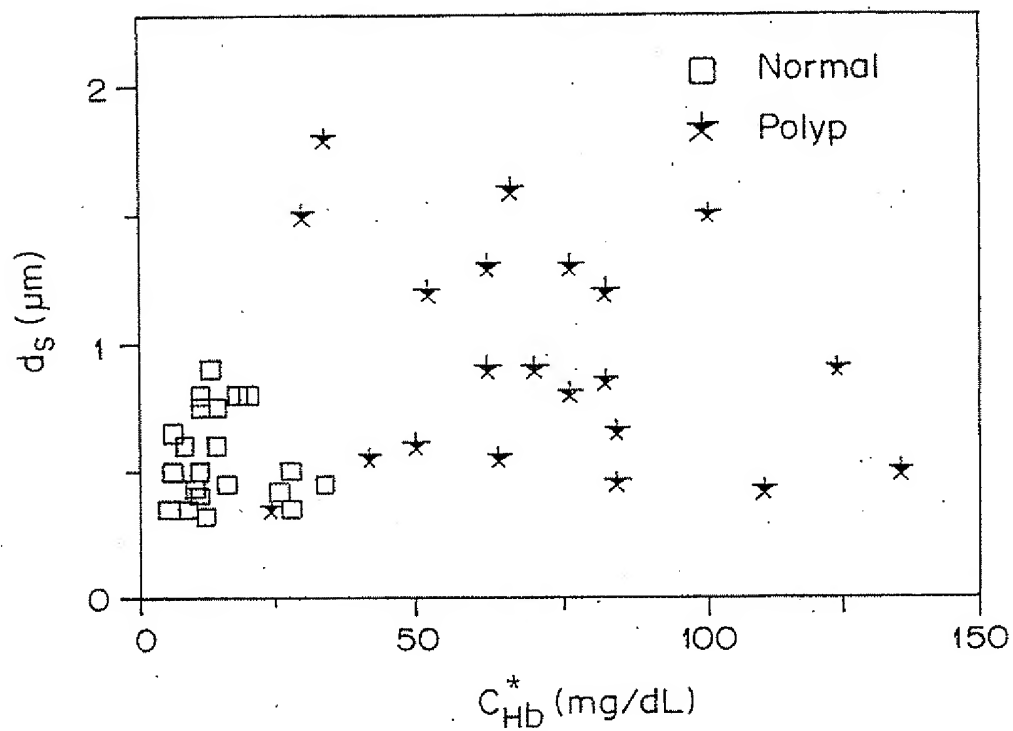
FIG. 15

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   A61B5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication. where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 718 417 A (C. KITTRELL ET AL.) 12 January 1988	1-4,7
X	see column 19, line 26 – column 20, line 11	9-12,15, 17,18,20
X	see column 24, line 4 – line 66	23,25,26
X	see figures 21-24	30,32-34
X	US 4 281 931 A (T. CHIKAMA) 4 August 1981	1-6
X	see column 1, line 9-15	9-14,17
X	see column 1, line 64 – column 2, line 7	18,20-22
X	see column 4, line 32 – column 5, line 17	24,30, 32-34
A		25,26,28

-/--

X	Further documents are listed in the continuation of box C.		X	Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance .

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 January 1999	01/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Rieb, K.D.

INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 14399 A (MIT) 3 September 1992	1-4,7
X	see page 2, line 4 - line 29	9-12,15
X	see page 8, line 16 - page 9, line 13	17-20,25
X	see page 18, line 23 - page 19, line 24	26,30-34
X	& US 5 280 788 A	
	cited in the application	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 4718417	A	12-01-1988	CA	1279901 A	05-02-1991
			CA	1339056 A	29-07-1997
			CA	1317641 A	11-05-1993
			CA	1329655 A	17-05-1994
			DK	130586 A	23-09-1986
			FI	861209 A	23-09-1986
			JP	2589674 B	12-03-1997
			JP	61257638 A	15-11-1986
			JP	2739933 B	15-04-1998
			JP	9117407 A	06-05-1997
US 4281931	A	04-08-1981	NONE		
WO 9214399	A	03-09-1992	US	5280788 A	25-01-1994
			EP	0575472 A	29-12-1993